

A transitional *Emiliana huxleyi* morphotype

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Abstract We recently published on the succession of two distinct morphotypes of *Emiliana huxleyi* (one heavily calcified, one more delicate form) during active upwelling offshore of Namibia (Henderiks *et al.*, 2012). Here, we describe a distinct morphotype (Type T, described herein) of *E. huxleyi*, which represents a transitional form between Type A and Type R. This morphotype is characterized by short and thick I-shaped distal shield elements and heavily calcified inner tube elements that extend irregularly into the central area.

Some coccospheres that showed coccoliths with merged I-elements can be considered an end-member form very closely resembling *E. huxleyi* Type R and *Reticulofenestra* spp. Type T dominated the phytoplankton community in mature upwelled waters offshore Namibia, immediately succeeding coastal-upwelling induced diatom blooms. Type T was encountered in a relatively restricted geographic area and may therefore provide a qualitative test of the reproductive isolation and/or phenotypic plasticity within natural populations of *E. huxleyi* morphotypes.

1. The ABC of morphotypes

The cosmopolitan coccolithophore species, *Emiliana huxleyi* (Lohmann) Hay and Mohler, inhabits tropical to polar waters, covering a wide range of oceanographic conditions (e.g. Brand, 1994; Cubillos *et al.* 2007). Its distribution is supported by high genetic diversity (Medlin *et al.* 1996; Iglesias-Rodriguez *et al.* 2006), as well as a high degree of morphological variability within and among natural populations (e.g. Hagino *et al.*, 2005; Hagino and Okada, 2006; Cubillos *et al.*, 2007). Six different morphotypes of *E. huxleyi* have been described based on different shape, size and degree of calcification of the coccoliths (Type A, B, B/C, C, R; Young *et al.*, 2003, and references therein and Type O; Hagino *et al.* 2011).

Young and Westbroek (1991) were the first to confirm genotypic variation between two different morphotypes (Type A and B) by testing immunological cross-reactions between the coccolith associated polysaccharide (CAP) of each type and antibodies to the CAP. Schroeder *et al.* (2005) identified a genetic marker for distinguishing strains of *E. huxleyi* Type A from strains of Type B. More recently, Cook *et al.* (2011) provided evidence for photosynthetic pigment and genetic differences between two main morphotypes found in the Southern Ocean, Type A and Type B/C. DNA sequencing of plastid gene *tufA* distinguished morphotypes A, B/C (indistinguishable from B), and R, while little variation was observed within morphotypes (Cook *et al.*, 2011). Minor, but consistent differences in mitochondrial (*cox1b-atp4*) gene sequences appear to be linked to the biogeography and temperature preference/tolerance (ecotypes) of different *E. huxleyi* strains (Hagino *et al.*, 2011). The two phylogenetic groups identified by these authors are both dominated by Type A strains (28 out of 39 tested), and therefore seem unrelated to coccolith morphology, despite tight sub-clustering of other tested morphotypes within the clades (3 strains of Type R and 4 strains of Type O, respectively). However, Hagino *et al.* (2011) did not classify Type A strains by degree of calcification, which is known to vary

between strains and to remain stable in culture, and which is suggestive of distinct calcification physiologies within this common morphotype.

It remains unknown whether different phenotypes and genotypes can occur within interbreeding populations, or whether different morphotypes represent reproductively isolated species. In culture, monoclonal strains (i.e. single genotypes) have never been reported to change from one morphotype to another, despite experimental evidence for significant phenotypic plasticity (i.e. a phenotypic change of a single genotype in response to changing environmental conditions) (Paasche, 2002; Langer *et al.* 2009). No reports of cross-breeding experiments exist – probably because it is not easy to induce the haploid stage within monoclonal diploid strains and other practical complications. However, the identification of "transitional" morphotypes in natural populations may provide qualitative evidence for the viability and/or frequency of cross-breeding between different morphotypes.

2. The succession of morphotypes

Two distinct morphotypes of *E. huxleyi* were found to be part of the phytoplankton succession during coastal upwelling offshore of Namibia (Henderiks *et al.*, 2012). Plankton sampling during cruise 48/5 of the R/V *Meteor* in October 2000 (Alheit, 2000; Figure 1) revealed that a heavily calcified *E. huxleyi* morphotype, herein described as Type T (but labeled as Type A* in Henderiks *et al.*, 2012; Plate 1d), was the dominant coccolithophore in mature upwelled waters immediately after exhaustion of diatom blooms that were found closer to shore. Type T occurred in high abundance with up to >1 million cells l⁻¹ at water depths between 0-50m. The highest cell concentrations were encountered at station 579, where they caused a chlorophyll and 19'-hexanoyloxyfucoxanthin maximum at ~17m depth. At the same time, a more delicate *E. huxleyi* morphotype (Type B/C; Plate 1a) dominated further offshore, in very late-succession stage upwelling and oceanic waters (Henderiks *et al.*, 2012). Here, we provide

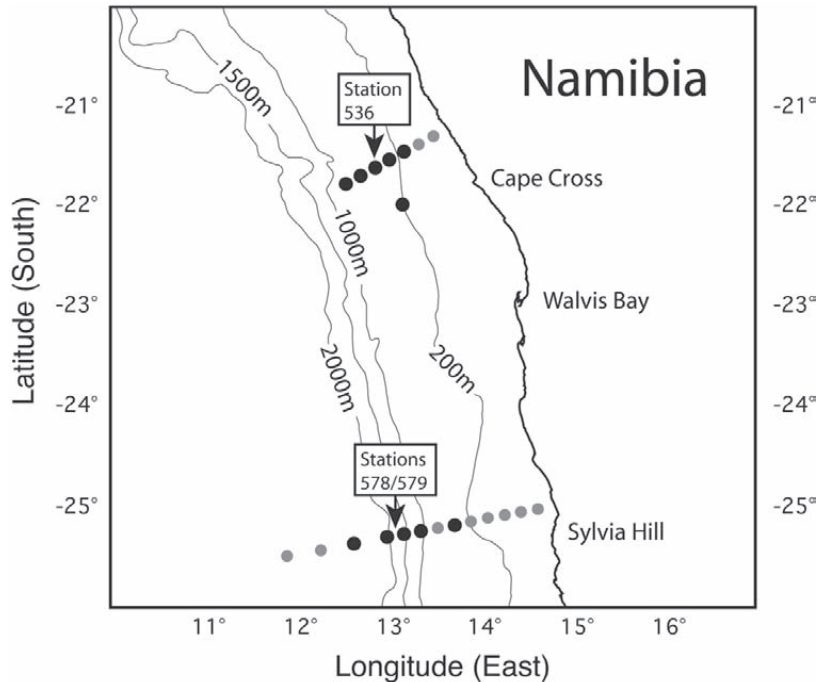


Figure 1: Stations sampled during Cruise 48/5 of the R/V *Meteor*, October 2000. Black symbols: *Emiliania huxleyi* populations (see Plate 1). Grey symbols: no coccolithophores.

additional photographic evidence for the observed morphological variation of *E. huxleyi* offshore Namibia (Plate 1) and describe Type T.

3. *Emiliania huxleyi* Type T

Coccoliths are medium sized (mean size 2.6-3.1 μm ; range 2-4 μm), with variable coccosphere diameters (mean diameter 4.9 μm ; range 3.4-8.4 μm). The distal shield elements are thick and short, with incomplete "hammer-head" terminations that we call "I"-elements, as opposed to the "T"-elements typical for *E. huxleyi* Type A (cf. Young *et al.*, 2003; Plate 1b). Often the distal shield I-elements are loosely arranged, because they are disconnected from neighboring elements at the outer margin, and they rarely extend beyond the proximal shield diameter. The inner tube cycle extends into the central area (CA) with irregularly shaped elements (similar to *E. huxleyi* Type R; Young *et al.*, 2003), which display variable degree of calcification, maximum thickness at the inner cycle and irregularly cover the CA, which has not been described for Type R (cf. Young *et al.*, 2003; but see a recent illustration of a heavily calcified Type R in Beaufort *et al.*, 2011). Type T also differs from Type R in that its distal shield elements are not as thick to appear merged together (but see rarely encountered 'combination' coccospheres that challenge this distinction; Plate 1e).

Type T coccoliths from offshore Namibia do not have the same smooth, over-calcified appearance of morphotypes observed in the upwelling region offshore Chile (Beaufort *et al.*, 2008; 2011), the heavily calcified forms that have been observed in the Bay of Biscaye under summer conditions (Beaufort & Heussner, 2001) or Type A "overcalcified" cf. Young *et al.* 2003. Instead, the presence of a gap between the distal shield elements and inner tube

element (Plate 1d,e) is a very consistent feature in the sampled populations. Young (1994) argued that such features are due to secondary dissolution of the central tube, and part of a spectrum from lightly etched to 'collapsed' coccoliths. No data on *in situ* pH were available for this study (see also discussion in Henderiks *et al.*, 2012), but it is interesting to note that the heavily calcified *E. huxleyi* morphotypes discovered in low-pH upwelling waters offshore Chile did not show such supposedly 'etched' features (Beaufort *et al.*, 2008; 2011).

We also note that, despite obvious differences in coccolith size and coccosphere diameter (compare Plate 1g), the CA of Type T is very similar to the CA of *Reticulofenestra parvula* var. *tecticentrum* (Okada & McIntyre 1977) Jordan & Young, 1990, an overcalcified form of *Reticulofenestra* in which the inner tube cycle closes the central area, without a central collar between the CA and distal shield elements.

Based on its irregular grill structure in the CA, its heavily calcified, but short and incomplete distal shield elements, as well as size (<4 μm), we conclude that *E. huxleyi* Type T is a transitional form on a morphological spectrum between Type A and Type R (compare classification in Hagino *et al.* (2011); their Table 1). Unfortunately, no cells were isolated and kept in culture, so we have not been able to test the phenotypic stability of this form. However, the morphological features of Type T were highly consistent within and between the sampled populations, while being distinctly different from the other *E. huxleyi* morphotypes that were encountered further offshore and later in the plankton succession (Plate 1a-c).

4. Concluding remarks

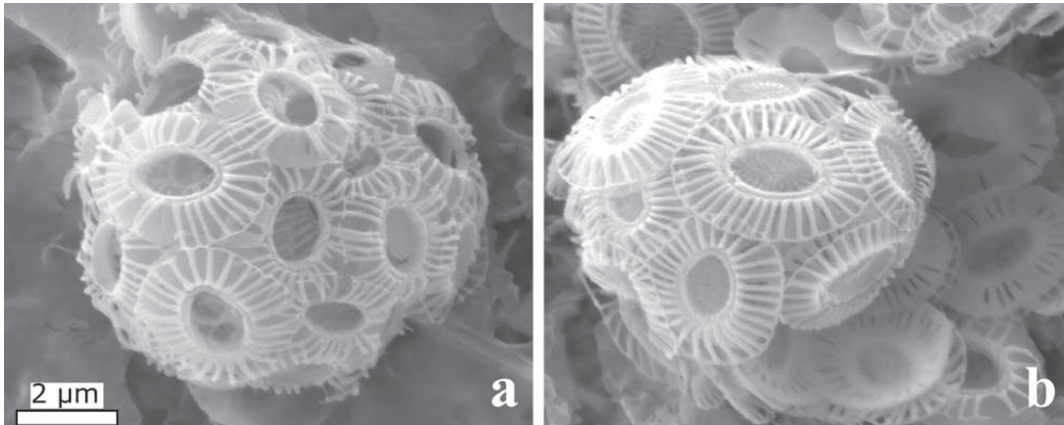
Over the past two decades, our research community has collected extensive evidence for distinct morphotypes, ecotypes and genotypes of *E. huxleyi*, in the quest to understand why this cosmopolitan taxon has a competitive edge over most other coccolithophores. Even if the relationship morphotype - ecotype - genotype is not necessarily a straightforward one (e.g. Langer *et al.*, 2009; Hagino *et al.*, 2011), it is very important to differentiate ("split") between morphotypes in natural samples, test strains for morphological stability/plasticity in the laboratory, and further develop a classification scheme that includes transitional forms between already established morphotypes.

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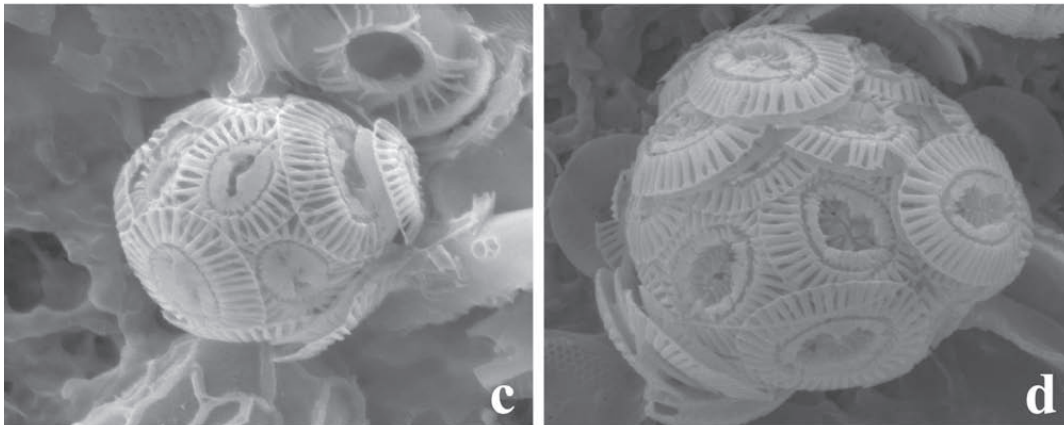
Plate 1

Scanning electron micrographs of *Emiliana huxleyi* morphotypes encountered offshore Namibia (cruise 48/5 of the R/V *Meteor* in October 2000). Scale bars: (a-e) = 2 μm , (f) = 1 μm .



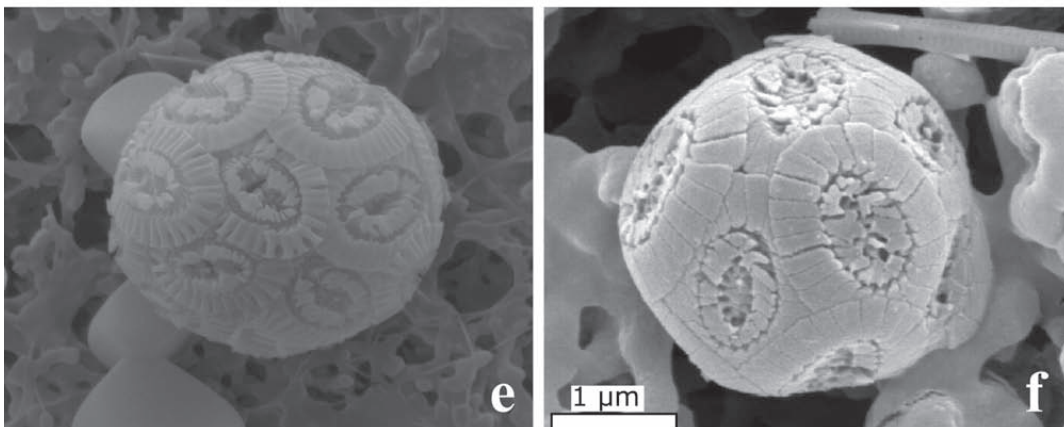
Emiliana huxleyi Type B/C, Stn 536 (surface)

E. huxleyi Type A, Stn 536 (surface)



E. huxleyi Type A 'overcalcified', Stn 536 (surface)

E. huxleyi Type T, Stn 579 (17m)



E. huxleyi Type T-R, Stn 578 (surface)

Reticulofenestra parvula var. *tecticentrum*
(image from Young et al. 2003)

- Type B/C coccosphere with double layer of coccoliths. Station 536 (surface).
- Type A coccosphere, rarely encountered in our samples. Station 536 (surface).
- 'over-calcified' Type A, nearly entirely covered central area, found to co-exist with Type B/C. Station 536 (surface).
- Type T coccosphere with double layering of coccoliths. Station 579 (chlorophyll maximum).
- Type T-R coccosphere, transitional form, merged distal shield elements (Type R-like) on some coccoliths, others as described for Type T, with short and thick I-elements. None of the distal elements connected by central collar. Station 578 (surface).
- Reticulofenestra parvula* var. *tecticentrum*, as illustrated in Young *et al.*, 2003 (image credits M. Geisen & J. Young). Inner tube cycle closing the CA irregularly, distal shield elements not connected by central collar. However, note scale bar = 1 μm ; coccoliths and coccosphere diameter of this specimen are significantly smaller than the illustrated *E. huxleyi* morphotypes.

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